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JARID1 Histone Demethylases: Emerging Targets in Cancer

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Abstract

JARID1 proteins are histone demethylases that both regulate normal cell fates during development and contribute to the epigenetic plasticity that underlies malignant transformation. This H3K4 demethylase family participates in multiple repressive transcriptional complexes at promoters and has broader regulatory effects on chromatin that remain ill-defined. There is growing understanding of the oncogenic and tumor suppressive functions of JARID1 proteins, which are contingent on cell context and the protein isoform. Their contributions to stem cell-like de-differentiation, tumor aggressiveness, and therapy resistance in cancer have sustained interest in the development of JARID1 inhibitors. Here we review the diverse and context-specific functions of the JARID1 proteins that may impact the utilization of emerging targeted inhibitors of this histone demethylase family in cancer therapy.

Keywords

JARID1A; JARID1B; JARID1C; JARID1D; KDM5; cancer epigenetics

Overview of JARID1 Family Function

Growing understanding of how tumors exploit the enzymatic machinery of chromatin regulation is opening opportunities for therapies targeting the **cancer epigenome** (see Glossary). Presently the only cancer drugs targeting chromatin regulators in clinical use are inhibitors of histone deacetylases (HDACs) and DNA methyltransferases, which have shown partial efficacy in certain malignancies. Other potential therapeutic targets include enzymes that define the methylation state of histones on lysine and arginine residues. These methylation events were considered irreversible until discovery of the first histone demethylase, LSD1 (*KDM1A*), a FAD-dependent amine oxidase specific to **H3K4me1/2** and H3K9me1/2 [1]. Subsequently, another class of demethylases emerged that acts upon

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methyllysine residues via an Fe²⁺ and 2-oxoglutarate-dependent mechanism. Many demethylases in this class not only regulate normal cell fates but have oncogenic and tumor suppressive functions. Prominent among them are the four members of the JARID1 (KDM5) family, which are implicated in numerous cancers and vary in nomenclature, developmental expression, and adult tissue distribution (Table 1). This family specifically demethylates H3K4me3 and H3K4me2 and is defined by conservation of multiple domains whose functional relationships are described in Box 1.

Box 1

JARID1 Structure-Function Relationships

JARID1 proteins contain multiple conserved domains that define their activity and substrate specificity [91] (Figure I). The catalytic core of these proteins contains a Jumonji domain, a motif shared by diverse proteins including most histone demethylases. The JARID1 family also shares a C₅HC₂ zinc finger that contributes to catalytic activity. JARID1 proteins are uniquely identified by separation of their catalytic cores into N and C terminal components (JmjN and JmjC) by an AT-rich interacting domain (ARID) and a PHD finger (PHD1). These inserted elements minimally impact catalytic activity based on their deletion having no effect on *in vitro* enzyme kinetics [92]. The ARID domain provides the main DNA-binding interface via its L1 loop and recognizes a GCACA/C consensus motif in the case of JARID1B [93], whereas PHD1 participates in histone tail recognition. JARID1B's PHD1 subunit binds only H3K4me0 [94] and thus may stabilize target gene repression after demethylation [95]. PHD1's interaction with H3K4me0 also allows removal of adjacent H3K4me3 marks, thus coupling the reader and catalytic domains in a feed forward loop that spreads demethylation on chromatin [96]; however, this mechanism may not apply to all isoforms based on JARID1D's PHD1 having much lower affinity for unmethylated H3K4. Similarly PHD3 has specificity for the H3K4me3 substrate [94] but is not essential for substrate recognition and is not conserved in JARID1C and JARID1D. Evolutionary conservation is evident from the JARID1 homologues present in *D. melanogaster* (Lid), *C. elegans* (Rbr-2), and *S. cerevisiae* (Jhd2), which all contain divided catalytic domains specific for H3K4me3/2. In yeast, absence of ARID and C₅HC₂ domains as well as the C-terminal PHD fingers suggest that these components evolved to facilitate cell fate decisions in multicellular organisms. Accordingly, it is mainly the C-terminal half of the mammalian forms that participate in the diverse protein complexes to dictate cell context-specific functions [2, 97–99].

Figure 1. Conservation of domain structure across JARID1 family members

Aligned structural motifs are shown for the four JARID1 isoforms, along with the single homologues present in *D. melanogaster*, *C. elegans*, and *S. cerevisiae*.

Before discovery of their histone demethylase function, the JARID1 isoforms were identified across diverse fields including stem cell biology and congenital disease, as detailed in Box 2. Their observed roles in cancer progression and therapy resistance have led to ongoing pursuit of inhibitors tailored to unique features of the JARID1 family's catalytic domain. Despite this effort, use of inhibitors is impaired by limited understanding of the biologic contributions of JARID1 demethylases to disease states. Herein, we review known functions of JARID1 family members in cancer and highlight the potential of targeting aspects of chromatin regulation mediated by these demethylases to improve cancer therapy.

Box 2

JARID1 proteins in normal development and congenital disease

JARID1A was discovered in 1991 in a cDNA expression library screen for interactors of pRb [100]. This interaction mediates Rb's ability to restrain cell cycle progression and promote differentiation by repressing E2F target genes [101]. JARID1C was identified as an X-linked gene that escapes X-inactivation during embryogenesis [102]. Shortly thereafter, JARID1C loss-of-function was found to be a major etiology of X-linked retardation [103]. JARID1D was originally reported as a minor histocompatibility antigen on the Y chromosome [104] and remains the least studied isoform. JARID1B was described last in 1999 using a screen of cDNA libraries from mammary epithelial cells upon c-ErbB2 signal activation [105]. Confirmation that JARID1B is upregulated by oncogenic signals in breast cancers initiated interest in its role in malignancy. Shared progress in these diverse fields accelerated after discovery that all isoforms act as H3K4me3/2-specific demethylases [3, 85, 86, 89, 90].

JARID1B or JARID1C deletion can be embryonic lethal, whereas JARID1A knockout mice appear normal [106, 107]. In embryogenesis, JARID1A and JARID1B have functions in activating the zygotic genome [108], and their expression subsequently peaks

at the blastocyst stage. Although JARID1B was once thought to be required for ESC self-renewal, this view is contradicted by JARID1B knockout producing normal or enhanced ESC self-renewal and marker expression [109]. Knockout ESCs instead show impaired neural lineage specification, a finding consistent with the neural defects in JARID1B knockout embryos [110]. A similar role for JARID1C is suggested by the brain-specific expression in Zebrafish embryos and neural defects following its knockdown [86]. JARID1 proteins are also implicated in later development and adult tissue homeostasis, including myogenic differentiation of embryonic fibroblasts [111]. A role for JARID1B in postnatal mammary gland development was observed in a viable strain of JARID1B knockout mice [33] and mice where only JARID1B's ARID domain was deleted [106]. The latter mice display a phenotype where mammary growth was delayed at puberty and early pregnancy. These findings coincide with the high JARID1B expression in a few adult tissues including testes and the pregnant mammary gland, whereas the other isoforms are more uniformly expressed. JARID1B appears to be a negative regulator of hematopoietic stem cell (HSC) activity and is required for HSC differentiation [112], although its function here remains controversial [113]. Roles for JARID1B have also been described in adult neural stem cell maintenance [114] and angiogenesis [116], and JARID1A participates in suppression of uterine contraction [115].

JARID1 Proteins in Cancer

Roles in Tumor Progression

Of the three JARID1 isoforms (JARID1A, B and C) upregulated in cancers, the relationship between JARID1B and tumorigenesis is the most studied thus far. JARID1B has been primarily regarded as an oncogene in breast cancer [3] despite some evidence for its overexpression suppressing invasion of MDA-MB-231 triple-negative breast cancer cells [2]. JARID1B amplification and overexpression are strongly associated with poor prognosis in hormone receptor-positive tumors and drive a luminal-type gene expression profile [4]. It is also upregulated in numerous other cancers including prostate [5], hepatocellular [6], and ovarian [7]. Despite low JARID1B levels in melanomas relative to benign nevi [8], these cancers contain a fraction of cells where high JARID1B expression confers stem cell-like molecular and functional traits [9], drives oxidative metabolism, and increases drug resistance [10]. Description of similar **JARID1B^{high}** cells within head and neck cancers [11] highlights JARID1B's contribution to aspects of **intra-tumor heterogeneity** and **epigenetic plasticity** that contribute to therapy resistance. JARID1A upregulation may also be advantageous in breast and head and neck cancers [12, 13] and was shown to mediate tumorigenesis in Rb^{-/+} and MEN1 null genetic mouse models of pancreatic islet cell tumors [14]. Furthermore, the C-terminal plant homeodomain (PHD) of JARID1A appears in an oncogenic translocation that fuses it to the NUP98 nuclear pore complex protein in acute myelogenous leukemia (AML) [15]. Lastly, upregulation of JARID1C is associated with poor prognosis in breast and prostate cancers [16, 17].

Roles in Tumor Suppression

JARID1C has a firmly established dual role as both an oncogene and a tumor suppressor. Evidence for the latter is apparent in clear cell renal cell carcinomas (CCRCCs) where sex-biased loss-of-function mutations in JARID1C may contribute to the predominance of this cancer in males [18, 19]. JARID1C may also be tumor-suppressive in human papilloma virus-related malignancies since it represses the E6 and E7 viral oncoprotein promoters in a complex with viral E2 [20]. JARID1B can also negatively regulate the malignant state mediated by genome-wide H3K4me3 hyper-methylation present in leukemias driven by the MLL methyltransferase [21]. Recently, JARID1D was shown to be a tumor suppressor based on its downregulation, mutation, or loss in prostate cancer [22] and CCRCC [23]. In hormone-sensitive prostate cancers, JARID1D directly interacts with the androgen receptor (AR) to attenuate the transcriptional activation of AR target genes. Subsequent loss of JARID1D expression in progression to castration-resistant disease may lead to treatment resistance by dysregulating AR signaling [24]. These diverse roles for JARID1 proteins in multiple cancer types underscore their high context dependence as potential cancer biomarkers and drug targets.

JARID1-Mediated Transcriptional Regulation

Transcriptional Repression

H3K4me3 is globally increased at promoters of transcribed genes and is widely regarded as an activating mark for transcription. This view is supported by H3K4me3 recruiting the pre-initiation factor TFIID to promoters of certain p53-regulated genes [25]. Thus the JARID1 proteins have mainly been studied as transcriptional repressors whose gene targets vary based on chromatin state and availability of binding partners. These partners include various chromatin-regulatory complexes (Box 3) that coordinate multiple repressive histone modifications (Figure 1A). Though H3K4me3 has been historically associated with transcriptional activation, continued rise in H3K4me3 after RNA transcription peaks [26] makes it unclear whether the mark is entirely instructive for transcription or also an effect of it [27]. Furthermore, loss of H3K4me3 does not affect transcription of many genes [28], suggesting that the transcriptional repressive function of the JARID1 family is targeted to only a subset of genes and not a uniform role across the genome.

Box 3

JARID1 interactions with other chromatin regulators

Target gene specificities of JARID1 demethylases are shaped by multiple interactions with repressive complexes containing other chromatin regulators (Figure 1A). JARID1A and JARID1B associate with Polycomb Repressive Complex 2 (PRC2), which contains the repressive H3K27 methyltransferase EZH2 [117, 118]. When complexed with PRC2 at retinoic acid (RA) responsive genes, JARID1B has opposing functions determined by the ligand-bound RA receptor. In absence of the receptor, JARID1B/PRC2 represses RA-responsive promoters, whereas its presence converts JARID1B to a transcriptional activator that antagonizes PRC2. JARID1A participates in at least two other chromatin-regulatory complexes, the SIN3B-containing deacetylase complex and the nucleosome

remodeling and deacetylase (NuRD) complex. In the NuRD complex, JARID1A regulates genes controlled by the complex's catalytic subunit CHD4, an ATP-dependent chromatin remodeler [119]. Interestingly the SIN3B complex also contains EMSY and ZNF131, which are oncogenic proteins often upregulated together in breast cancer [120]. JARID1B also associates with the NuRD complex and the H3K4me1/2-specific demethylase LSD1 [2]. Inclusion of LSD1 and JARID1B in multiple complexes likely coordinates sequential removal of all three H3K4 methyl groups [94]. Finally, JARID1C interacts with the repressive H3K9 and H3K27 methyltransferase G9a in complex with HDACs and REST, which is a repressor of differentiation in neural progenitors [121].

Interactions of JARID1 proteins with the HDACs contained in the NuRD, SIN3B, and G9a complexes have potential therapeutic significance. Interplay between JARID1A and HDACs underlies a drug tolerant state spontaneously acquired by cell fractions within cancer cell lines [122]. This state was mediated by JARID1A but surprisingly could be depleted by HDAC inhibition. Cooperative HDAC/JARID1 interactions are further evidenced by JARID1A depletion causing a global increase of histone acetylation along with radiosensitization [66]. Insight into such effects was provided by HDAC inhibition increasing global H3K4me3 while suppressing JARID1 demethylase transcription by downregulating Sp1 [123]. Furthermore, HDACs directly promote H3K4 demethylation through prior removal of histone acetylation, which is known to impair substrate recognition by the yeast JARID1 homologue Jhd2 [124]. The interface between JARID1B and HDACs may also have an antagonistic aspect based on evidence for mutually repressive effects on each other's promoters [125–127]. There is also evidence for JARID1 proteins interacting directly with HDACs to mediate their recruitment [128]. These diverse interactions create opportunities to evaluate combinations of emerging JARID1 inhibitors with agents targeting other chromatin regulators including HDACs, which have established drugs in clinical use.

Other Transcriptional Regulatory Effects

In addition to direct transcriptional repression by H3K4 demethylation at promoters, JARID1 proteins exert gene regulatory effects by limiting enrichment of H3K4me3 in other regions of the genome. Such effects are best studied in embryonic stem cells (ESCs), where JARID1B regulates genes associated with self-renewal. Here JARID1B was shown to deplete H3K4me3 at intragenic sites marked by H3K36me3, where it was recruited by the chromodomain protein MRG15. This action increased gene expression, suggesting that JARID1B maintains a gradient of H3K4me3 between promoters and intragenic sites needed to repress **cryptic intragenic transcription** during expression of self-renewal genes [29]. This finding was extended by data showing that JARID1B-mediated H3K4me3 demethylation also occurs just outside the boundaries of active enhancers, which are characterized by the presence of H3K4me1 (Figure 1B). Silencing JARID1B reduced H3K4me3 at promoters of self-renewal genes and spread H3K4 methylation to gene bodies and enhancer boundaries while promoting expression of lineage-specific genes [30]. Similarly, JARID1C can regulate the ratio of H3K4me1 to H3K4me3 at both enhancers and super-enhancers to modulate their levels of activity [31, 32].

JARID1 proteins exert a variety of other transcriptional regulatory effects, including actions at promoters that activate transcription (Figure 1C). For instance, JARID1B deficiency in pubescent mice impaired expression of multiple regulators of mammary morphogenesis including FOXA1. Here JARID1B was required to recruit GATA3 to the FOXA1 promoter in order to activate transcription, although the demethylase dependence of this effect is unclear [33]. JARID1 demethylases may also stabilize active transcription by mediating cyclic turnover of H3K4me3 levels. In this role, the drosophila homolog Lid was shown to reset chromatin to an unmethylated state that allows a new cycle of methylation to sustain activity of the transcriptional complex [34]. Similarly, coordinated methylation and demethylation of H3K4me3 in yeast by the homolog Jhd2 has been shown to facilitate nucleosomal turnover in order to promote RNA Polymerase II (RNAPII) progression through coding regions [34]. Finally, there are emerging links between JARID1 proteins and other aspects of mRNA regulation including regulation of 3' UTR length [35] as well as differential splicing. JARID1B depletion near alternatively spliced exons broadened H3K4me3 peaks in those domains, leading to new splice events that diversified the mRNA repertoire during ESC differentiation [36].

Demethylase-Independent Effects

The first evidence of demethylase-independent JARID1 function was provided by a catalytically inactive form of Lid that rescued the embryonic lethality of its deletion mutant [37]. Lid has also been shown to promote gene transcription by diverse demethylase-independent mechanisms, including inhibition of the HDAC Rpd3 [38] and recruitment of the transcription factor Foxo to promoters of oxidative stress resistance genes [39]. Lid also regulates gene sets involved in mitochondrial structure and function [40] and reorganization of chromatin during meiotic prophase I [41]. Despite structural conservation, little information exists on retention of such demethylase-independent functions in vertebrates. One such function is described for JARID1A, which enhances the cyclic transcription in complex with the circadian rhythm-regulatory transcription factors CLOCK and BMAL1 (Figure 1C). This interaction allows JARID1A to increase Per2 promoter transcription in a demethylase-independent manner by inhibiting histone deacetylase (HDAC)-mediated chromatin condensation [42].

JARID1 Contribution to Cancer Progression

Cell Cycle Progression

JARID1A and JARID1B can promote G₁-S progression despite their paradoxical ability to maintain G₀/G₁ arrest in senescent fibroblasts by suppressing pRb target genes [43, 44]. Both isoforms increase Cyclin D1 levels and promote cell cycle progression, possibly through repression of the let-7e microRNA [45, 46]. Repression of the p27 promoter is another JARID1B-mediated mechanism of G₁-S progression that was first observed in normal embryos [47]. This effect is maintained in hepatocellular cancer [6] and is also described in lung cancers via JARID1A [46]. JARID1A and JARID1B-mediated repression of other cdk inhibitors is also reported, including effects on p15, p16, and p21 [6, 12, 48, 49]. It remains unclear what contextual determinants dictate whether JARID1 proteins drive G₁-S progression or promote arrest and senescence.

Activation of the PI3K Pathway

Interactions of JARID1 proteins with oncogenic signaling are best studied in relation to the PI3K/Akt pathway. JARID1B was recently implicated in activating Akt in hepatocellular carcinoma and hypopharyngeal squamous cell carcinoma via direct suppression of the promoters for the PTEN [50] and SHIP1 [51] phosphatases, respectively, thus providing a new basis for the epigenetic suppression of PTEN widely observed in cancer. Notably, ectopic Akt activation also increases JARID1B levels in oral cancer cells, suggesting that the PI3K pathway itself drives JARID1B upregulation [11] by presently unknown mechanisms. When combined with JARID1B-mediated PTEN repression, this finding predicts a feed forward loop of increasing Akt activation [52] that may explain the pathway hyperactivity found in tumor cells with highest JARID1B levels. An opposing interaction with PI3K signals has been described for JARID1A, whose direct phosphorylation by AKT leads to its exclusion from the nucleus of breast cancer cells and resulting inability to demethylate its histone substrate. Accordingly, the decreased expression of cell cycle-related genes caused by Akt inhibition was blunted by silencing JARID1A [53]. Notably, JARID1B lacks the Akt target consensus motif that is conserved in JARID1A across species. Together these observations suggest that a shift from JARID1A to JARID1B-mediated chromatin-regulatory effects occurs during PI3K/Akt activation.

EMT, Invasion, and Metastasis

JARID1A, JARID1B, and JARID1C have been shown to promote epithelial to mesenchymal transition (EMT) along with invasion and metastasis. JARID1B's augmentation of PI3K/Akt signaling in hepatocellular carcinoma cells indirectly connects it to the EMT-promoting effects that occur downstream of Akt activation [50]. More direct effects may arise from JARID1B repressing the promoters of both miR-200 family transcripts, which are potent negative regulators of EMT and maintain E-cadherin expression by suppressing Zeb1 and Zeb2 [54]. Accordingly, JARID1B target gene sets were enriched during TGF β -induced EMT in a KRAS-mutant lung cancer model [55]. Other microRNA-mediated effects of JARID1B include interactions with the breast cancer oncogene EMSY in repressing miR-31, a metastasis-associated microRNA and modulator of invasion [56]. JARID1C may exert similar effects in breast cancer, where its expression positively correlates with metastasis. Here it can promote cell migration and invasion by repressing the promoter for Breast Cancer Metastasis Suppressor 1 (BRMS1) [16]. High JARID1C levels in hepatocellular carcinoma cells were also shown to promote invasion and metastasis via repression of BMP7 [57]. Multiple studies on JARID1A have reported similar links with EMT, metastasis, and stem cell-like features [46, 58, 59]. Collectively these observations implicate actions of JARID1 proteins as a new basis for the known association between stem cell-like traits and aggressive, mesenchymal features in carcinomas.

Adaptation to Hypoxia

A hypoxic microenvironment exerts opposing effects on JARID1 proteins by promoting expression while impairing demethylase function. JARID1B levels rise in a HIF1 α -dependent manner under hypoxic conditions [10, 60], which also deprive JARID1 demethylases of O₂ as a catalytic substrate. Accordingly, hypoxia blocked JARID1B's

repression of promoters for genes regulated by the androgen receptor in prostate cancer cells [61]. Low O₂ was similarly shown to block JARID1A's demethylase-dependent repression of promoters [62, 63], although this isoform does not appear susceptible to HIF1 α -dependent effects [60]. Regardless, increases in all the isoforms have been reported under hypoxia [64] and may primarily be an adaptive response for maintaining their normoxic functions [63]. However, hypoxia could hypothetically also mediate a shift toward demethylase-independent gene regulatory effects that support tumor progression in a hypoxic milieu.

Control of Genomic Instability

New evidence for JARID1 proteins participating in the DNA damage response adds an intriguing dimension to the diverse functions of the family of proteins. JARID1 proteins were first linked to DNA damage when JARID1A was shown to accumulate at γ -H2AX foci and decrease H3K4me3 at those sites after irradiation [65]. However, an increase in radiation sensitivity seen after JARID1A depletion in HeLa cells did not result from deficient double-stranded break repair [66]. JARID1B was implicated in repair of double strand breaks after its loss was shown to promote spontaneous DNA damage and sensitize both osteosarcoma and breast cancer cell lines to genotoxic insult. Here JARID1B was enriched at DNA damage sites, where it was required for recruiting Ku70 and BRCA1 during non-homologous end joining [67]. Similar findings have also implicated JARID1C in enforcing genomic stability in sporadic renal cancer [68].

These functions of JARID1 proteins in DNA damage have potential to shed light on features observed in the fraction of melanoma and oral cancer cells expressing the highest levels of JARID1B. These highly tumorigenic JARID1B^{high} cells are slow-cycling [9, 52] and yet display a cell cycle distribution shifted toward G₂/M [11]. This distribution could arise from G₂/M checkpoint activation protecting genome integrity in this stem cell-like population and contribute to the observed resistance of JARID1B^{high} cells to DNA damaging drugs [10]. This finding may also relate mechanistically to recent observations in pluripotency, where prolonging S/G₂ intrinsically favors stem cell maintenance [69]. Loss of such checkpoint activation might also explain why silencing JARID1B in melanoma causes rapid *in vivo* growth followed by proliferative exhaustion [9].

Although the reason for the G₂/M expansion observed in JARID1B^{high} cells remains unclear, this effect temporally corresponds to a post-translational modification of JARID1B. During G₂/M phase, there is a peak in both JARID1B levels and its SUMOylated form, after which JARID1B target gene occupancy decreases and JARID1B degradation increases [70]. DNA damage induced SUMO-2 conjugation to both JARID1B and JARID1C, which caused JARID1B degradation but promoted recruitment of JARID1C to chromatin in the osteosarcoma cancer cell line U2OS [71]. These early findings hint at mechanisms underlying a poorly characterized interface between cell cycle progression and control of genomic instability that is regulated by JARID1 proteins in cancer.

Targeting JARID1 Function

Small molecule inhibitors of JARID1 proteins are actively being pursued as anti-cancer therapeutics [72] and for treatment of certain nonmalignant diseases [73]. Early inhibition strategies used Fe^{2+} chelation or competitive 2-oxoglutarate analogs to impede the catalytic mechanism that JARID1 proteins share with many histone demethylases. Developing inhibitors with improved specificity has been hindered by the close structural homology and hybrid features of the JARID1 catalytic core with the KDM4 and KDM6 families [74]. This limitation explains the lack of specificity of early inhibitors such as 2,4-PDCA [75], PBIT [76], and hydroxamate compounds [77]. A nonselective inhibitory mechanism based on the ability of disulfiram to inhibit JARID1A's PHD3 binding to H3K4me3 has also been described and used to inhibit growth of AMLs driven by a NUP98-JARID1A fusion gene [78]. Recent structural analyses identifying amino acid side chains and conformational plasticity unique to the JARID1 active sites have facilitated improvements in potency and selectivity of inhibitors [79, 80]. Presently the most notable result is CPI-455, a prototype tool compound with 200-fold selectivity for JARID1 over KDM4 demethylases and at least 500-fold selectivity over other KDM families [81]. 1,7-naphthyridones are a second recent example of a small molecule class with potential JARID1 family specificity [82]. Two other lead compounds have been described with selectivity for the JARID1A isoform [83, 84]. In general, biologic effects of these JARID1 inhibitors *in vitro* appear fairly modest. However, their effects in tumor and host, either as a single agent or in combinatorial use, are largely unknown and difficult to anticipate given the diverse, context-specific roles of these large multi-function proteins. The contextual basis of these effects may be determined in large part by associations with larger chromatin regulatory complexes detailed in Box 3.

Concluding Remarks

Interest in the role of JARID1 proteins in development and cancer has increased steadily since 2007, when they were recognized as H3K4 demethylases [3, 85–90]. This interest is sustained partly by the observation that upregulation of these chromatin regulators can mediate a cancer cell state characterized by stem cell-like features, aggressive invasion and metastasis, and therapy resistance. The mechanisms by which JARID1 proteins contribute to cancer progression overlap with those in development but also likely include gene-regulatory actions unique to malignancy. To date, work to define these mechanisms in cancer has mainly considered demethylase-dependent repression of specific promoters and not the broader range of potential gene regulatory effects, including demethylase independent ones. Actions of JARID1 proteins in cancer that are potentially targetable by demethylase inhibition are highlighted in Figure 2. Determining which effects are demethylase-dependent is needed to clarify the features of cancers that are targetable via JARID1 enzymatic activity (see Outstanding Questions), and this effort is made timely by recent emergence of potent tool drugs with selectivity for the JARID1 family. In addition, the diverse binding partners that dictate the gene specificity of JARID1 proteins underscore their cell context dependence as drug targets and highlight the need to identify molecular subgroups of tumors where targeting them has relevance. Despite these caveats, targeting the JARID1 family and other histone modifiers holds promise in combination therapies designed to address the epigenetic plasticity that allows malignant cells to escape current cancer drugs. Clarifying the interface

of JARID1 family with the DNA damage response may guide application of these inhibitors with numerous cytotoxic or targeted drugs impacting this process. Similarly, combining JARID1 inhibitors with select targeted agents that inhibit oncogenic signaling pathways may address the epigenetic plasticity that can prevent such modern drugs from achieving durable cancer control.

Outstanding Questions Box

- Roles of JARID1 proteins beyond direct promoter repression remain poorly defined and include actions that activate transcription and demethylase-independent functions.
- Deprivation of the JARID1 family's O₂ catalytic substrate during tumor hypoxia predicts a shift toward demethylase-independent effects whose biologic and therapeutic implications are not known.
- Understanding the molecular determinants of whether JARID1 proteins drive G₁-S progression or promote differentiation and senescence would guide more effective therapeutic application of JARID1 inhibition.
- Determining detailed interactions of JARID1 proteins with oncogenic signaling pathways like PI3K/Akt might facilitate rational combination of JARID1 inhibitors with existing targeted agents.
- Clarifying the interface of JARID1 family with the G₂/M checkpoint and DNA damage repair may guide combinatorial application of JARID1 inhibitors with other agents impacting these processes.
- The diverse binding partners that dictate cell context-specific gene regulation by JARID1 proteins underscore a need to identify the molecular subgroups of cancers where JARID1 targeting has clinical relevance.
- Defining the detailed roles of JARID1 proteins in repressive complexes containing other histone modifiers will facilitate combinatorial use of agents targeting other chromatin regulators, including established HDAC inhibitors.

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Glossary box

Cancer epigenome

The genome-wide chromatin landscape including DNA methylation and histone posttranslational modifications, including methylation, acetylation, phosphorylation, and ubiquitination that contribute to malignant cell states.

H3K4me0/1/2/3

The four methylation states of lysine 4 in Histone H3. The number represents number of methyl groups added to the ε-amine in the lysine side chain.

JARID1B^{high} cells

A minority cell fraction within cancer cell lines that has elevated JARID1B mRNA and protein levels and was purified using J1BpromEGFP, a promoter-based fluorescent reporter of JARID1B expression [9].

Intra-tumor heterogeneity

Cellular diversity in a given tumor, comprised of diversity among tumor cells defined by both genetic and epigenetic differences, as well as presence of multiple stromal cell types that impact the microenvironment.

Epigenetic plasticity

Durable shifts in cell phenotype that occur through reversible changes in chromatin and may recapitulate aspects of cell state transitions that occur during normal development and tissue homeostasis.

Cryptic intragenic transcription

Transcription that initiates within the coding region of a gene. This event is associated with the failure to re-condense chromatin behind the transcriptional elongation complex and decreased transcription of the full-length gene.

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Trends Box

- The epigenetic plasticity that allows a subset of cancer cells to escape current drugs has encouraged pursuit of therapies targeting chromatin regulation.
- The JARID1 protein family's emerging roles in cancer progression and therapy resistance have motivated development of small molecule inhibitors with improving potency and specificity.
- The therapeutic relevance of targeting the JARID1 family is suggested by its multiple roles in cell cycle regulation, oncogenic signaling, hypoxia adaptation, and control of genomic instability.
- Improving understanding of the diverse actions of JARID1 proteins in development and malignancy are facilitating effective application of targeted inhibitors in relevant therapeutic contexts.
- Detection of demethylase-independent effects of JARID1 proteins indicates that a subset of their functions are not targetable with catalytic inhibitors.

Figure 1. Categories of gene-regulatory effects mediated by the JARID1 family

(A) JARID1 proteins (blue) participate in transcriptional repression by removing methyl groups (Me) from H3K4 at gene promoters (left). Gene specificity is partly dictated by the identity of the repressive complex where JARID1 proteins participate (right). Many such complexes contain other chromatin modifiers including histone methyltransferases (HMTs, red) that generate repressive methyl marks, HDACs (orange) that remove acetyl groups (Ac), and the H3K4me1/2 demethylase LSD1 (green). (B) JARID1 demethylases also promote transcription by regulating H3K4 methylation across broader regions of chromatin. The illustrated example shows JARID1 enforcing the H3K4me1 mark (red) that promotes enhancer activity while suppressing cryptic intragenic transcriptional initiation by preventing the spread of H3K4me3 (green) to gene bodies. (C) JARID1 proteins participate in demethylase-independent transcriptional activation through the recruitment of transcription factors (TF, right) to promoters or antagonizing HDAC function. JARID1A-mediated demethylase-independent effects have been observed in this setting. * Demethylase independence of GATA recruitment by JARID1B has not been formally demonstrated.

Figure 2. A subset of the JARID1 family's actions in cancer progression are potentially targetable

Cell cycle progression: JARID1A and JARID1B promote G₁-S progression through repression of CDK inhibitors and Cyclin D upregulation. *PI3K pathway activation:* JARID1B's repression of the PTEN and SHIP1 phosphatases can enhance activation of the PI3K/Akt pathway. Akt activation increases JARID1B expression indicating a putative feed forward loop of increasing PI3K activity. By contrast, phosphorylation of JARID1A as a direct Akt substrate excludes it from its actions in the nucleus as a result of PI3K signaling. *EMT, invasion, and metastasis:* JARID1A, JARID1B, and JARID1C are all linked to promotion of these effects by multiple mechanisms. *Hypoxia:* JARID1B is a direct HIF1 α target gene that is upregulated by hypoxia, and similar HIF1 α -independent effects are reported for JARID1A and JARID1C. At the same time, O₂ substrate deprivation reduces the catalytic actions of all isoforms but also may produce a qualitative shift toward demethylase-independent effects. *Excessive genomic instability:* JARID1B and JARID1C can participate directly in the DNA damage response and may prevent exhaustion of a stem cell-like tumor cell fraction by limiting excess cancer genome instability and conferring resistance to DNA-damaging drugs. *Tumor promoting effects that are potentially targetable by JARID1 inhibition are indicated by bold lines.*

Table 1

Nomenclature and normal expression pattern of JARID1 isoforms

Nomenclature		Expression			References
Common	Aliases	Development	Adult Tissue		
JARID1A KDM5A	RBP2 RBBP2	<ul style="list-style-type: none">• Viable and fertile null mutant	<ul style="list-style-type: none">• Ubiquitous	[72, 106]	
JARID1B KDM5B	PLU1 CT31 PUT1 PPP1R98 RBP2-H1 RBBP2H1A	<ul style="list-style-type: none">• Variability in null mutant phenotype<ul style="list-style-type: none">– Embryonic lethal– Viable with severe developmental defects• Dynamic spatial and temporal expression in multiple tissues	<ul style="list-style-type: none">• High expression in testis• Moderate expression in eye, prostate, ovary• Upregulated in developing mammary during pregnancy	[33, 106, 129]	
JARID1C KDM5C	SMCX MRXJ; MRXSJ; MRXSJC; MRX13 XE169 DXS1272E	<ul style="list-style-type: none">• Embryonic lethal null mutant• Upregulated in developing neural tissues, liver, and heart	<ul style="list-style-type: none">• Ubiquitous	[86, 103, 107, 130]	
JARID1D KDM5D	SMCY HY; HYA; KIAA0234	<ul style="list-style-type: none">• Limited data	<ul style="list-style-type: none">• Increased in pancreas, lung, brain, and skeletal muscle	[103]	